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Methods for Predicting Chronic Toxicity Parameters of Substances in the Area of Water Hygiene

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The development of rapid methods for determining chronic toxicity parameters of chemical compounds introduced by the oral route into an organism with water was studied.

Formulas are verified with which threshold values and no-effect toxicity doses for substances can be obtained on the basis of various toxicologic parameters. For the example of organophosphates, a methodological scheme was developed for conducting an accelerated experiment for predicting the chronic toxicity of low and average cumulative compounds. Results are presented indicating the inadvisability of using lower animals as test subjects with the aim of predicting toxicity for higher animals. It is pointed out that patterns of homology and isomerism are observed in the comparison of chronic intoxication threshold doses as well as in acute toxicity values of substances.

The use each year of hundreds of diverse chemical compounds in industry and agriculture requires not only that permissible environmental levels be set for them but also that rapid methods of developing precise, reliable standards for chemical substances be found. An intensification of scientific research toward these ends is possible only if computational, evaluation, accelerated experimental and other methods are developed to determine the parameters of chronic toxicity of substances, particularly since current methods for evaluating health and toxicological aspects are time-consuming and cumbersome. This is illustrated by the fact that during a period of over 25 years, our country's science laboratories have developed only 600 health standards for the air of industrial shops, 200 for the ambient air, 400 for water, and about 50 for food products.

In our opinion, rapid methods of ascertaining threshold and no-effect levels of substances can most advantageously be used in setting health standards for chemical compounds in water. In this case the maximum permissible concentrations are established in accordance with three limiting factors: the organoleptic indicator, impact on the sanitary condition of the reservoir, and the toxicologic indicator. This contributes to successful application of rapid methods of determining the parameters of chronic toxicity of substances in water, since the time required to obtain threshold concentrations under the first two indicators is negligible. In addition, these two indicators are the principal ones in the setting of health standards for more than half of the substances studied.

Rapid methods for evaluating the toxicity of substances are especially useful in setting standards for new chemicals which have a marked effect on the organoleptic properties of water. Such methods are also of value in verifying and refining maximally permissible concentrations as defined in earlier experiments, and in accurately and objectively selecting dosages for planning chronic experiments.

The most promising areas for the development of rapid methods for predicting the parameters of chronic toxicity of substances are the following: (1) developing equations from the mathematical relationships between various indicators of the toxicity of substances (for a given class of chemicals) as

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determined in preliminary or long-term experiments used in setting environmental health standards; (2) developing equations for predicting toxicity on the basis of physical and chemical properties of the substances, values of the bonding energy of the chemical compounds, electron density distribution within the molecule, etc.; (3) developing methods for conducting rapid experiments that can predict the results of chronic experiments; (4) developing ways of predicting the toxicity of substances through use of simple biological test subjects, i.e., tissue cultures, daphnia, water saprophytes, fish, etc.; (5) investigating the possibilities of predicting the parameters of chronic toxicity of substances by using the principles of homology and isomerism.

We investigated these major areas in our research.

Equations Relating Toxicity Indicators

A number of equations were derived from earlier research by establishing correlations between limiting quantities discovered in setting standards for 300 substances in reservoir water and other health standards and physical and chemical properties. Analysis of the correlations indicates that the most accurate numerical values for the chronic toxicity of substances were obtained by using toxicological parameters such as lethal dose, lethal concentration, and maximal permissible concentration (LD_{50}, LC_{50}, $MPC_{workplace}$) (Fig. 1). The application of the so-called "physicochemical indices" of substances (solubility, specific weight, molecular weight, surface tension, etc.). was less useful in this regard.

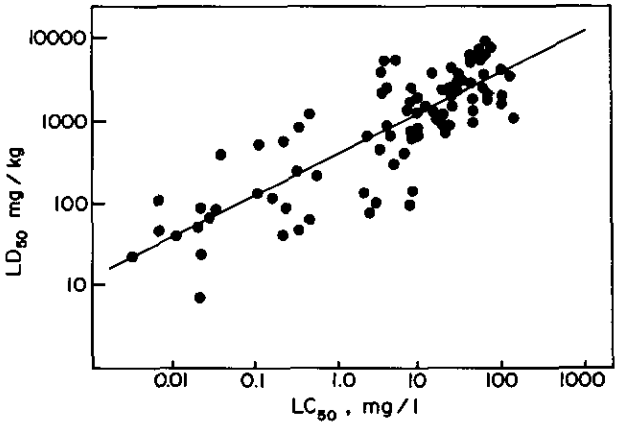

FIGURE 1. Correlation between LD_{50} and LC_{50} for chemical compounds. The line is for log LD_{50} = 0.5 log LC_{50} + 2.55.

In our most recent research along these lines we sought to apply more reliable criteria for obtaining accurate numerical values. The analysis included data on the threshold and no-effect levels of chronic toxicity of all of the substances studied, and of individual groups as well (organophosphates and organochlorine compounds). The data used were those obtained in setting health standards in water, indicators of acute toxicity, the maximum permissible concentrations in the air of workplaces, and threshold and no-effect concentrations of chronic toxicity with respect to the health standards in the ambient air of the chemicals studied. The data were analyzed mathematically by computer, by use of correlation and regression analysis, which yielded correlation coefficients and regression equations for the classes of chemicals studied [eqs. (1)–(17)].

For all substances:

$$\log TD = 0.65 \log TC - 0.37 \quad (1)$$

$$\log MID = 0.52 \log MIC - 0.88 \quad (2)$$

For organophosphate compounds (Figs. 2 and3):

$$\log TD = 0.99 \log MPC + 0.60 \quad (3)$$

$$\log MID = 1.099 \log MPC - 0.599 \quad (4)$$

$$\log TD = 0.91 \log LD_{50} - 2.0 \quad (5)$$

$$\log MID = 0.76 \log LD_{50} - 3.66 \quad (6)$$

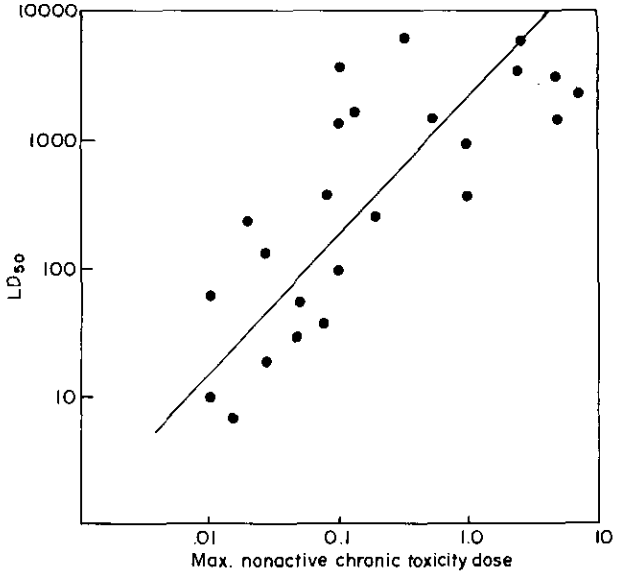

FIGURE 2. Correlation between LD_{50} of substances and the maximally inactive chronic toxicity dose (MID) for water. The line is for log MID = 0.76 log LD_{50} – 3.66.

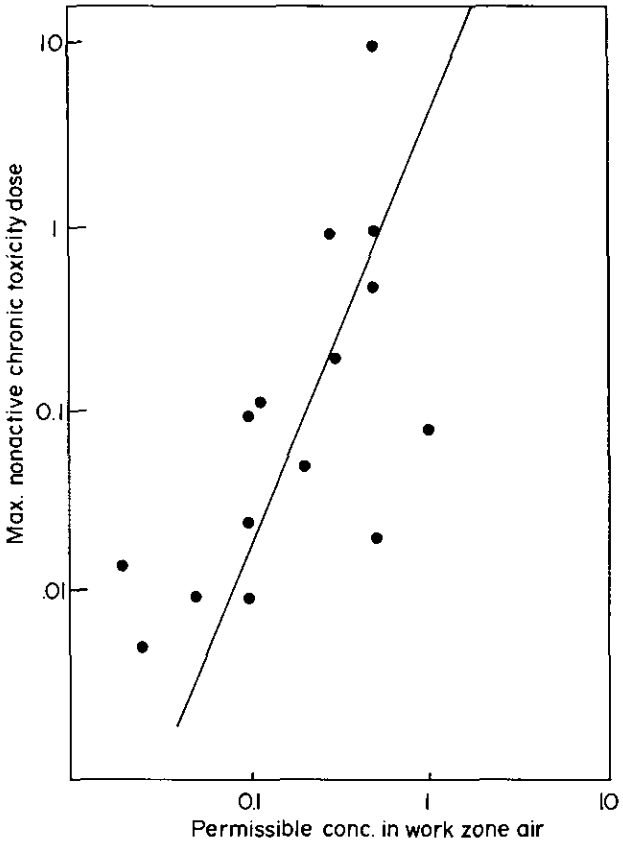

FIGURE 3. Correlation between the maximally nonactive chronic toxicity dose (MID) substances for water and maximal permissible concentration (MPC) in work zone air. The line is for log MID = 1.099 log MPC − 0.599.

For phosphoroorganic compounds:

$$\log \text{CTTD} = 0.99 \log \text{MPC} + 0.600 \quad (7)$$

$$\log \text{MNCTD} = 1.099 \log \text{MPC} - 0.599 \quad (8)$$

$$\log \text{CTTD} = 0.91 \log LD_{50} - 2.001 \quad (9)$$

$$\log \text{MNCTD} = 0.76 \log LD_{50} - 3.660 \quad (10)$$

For chloroorganic compounds:

$$\log TD = 0.438 \log \text{MPC} + 1.332 \quad (11)$$

$$\log TD = 1.28 \log LD_{50} - 4.54 \quad (12)$$

$$\log \text{MID} = 1.16 \log LD_{50} - 5.495 \quad (13)$$

For chloroorganic compounds:

$$\log \text{CTTD} = 0.438 \log \text{MPC} - 1.332 \quad (15)$$

$$\log \text{CTTD} = 1.28 \log LD_{50} - 4.541 \quad (16)$$

$$\log \text{MNCTD} = 1.16 \log - 5.495 \quad (17)$$

Here TD is the threshold dose, TC is the threshold concentration, MD is the maximum ineffective dose; MIC is the maximum ineffective concentration, CTTD is the chronic toxicity threshold dose; MNCTD is the maximally nonactive chronic toxicity dose; MPC is the maximum permissible concentration in the air of the work zone; LD_{50} is the median lethal dose.

Upon analysis of the material presented, it appears that application of regression equations for the combined classes of substances (140 compounds representitive of nitro compounds, amino compounds, and organophosphate and organochlorine compounds) lacks precision in predicting parameters of chronic toxicity, since the correlation coefficients of the classes of compounds investigated are very low (0.3–0.4). Determination of the parameters of chronic toxicity of organochlorine compounds (TD and MID) from the values of their average lethal doses (LD_{50}), and of the parameters of chronic toxicity of phosphoroorganic compounds (TD and MID) from the MPC in the work zones may be more accurate, since the correlation coefficients for these groups are 0.6–0.7.

Results from the experimental determination of threshold and no-effect doses as established by recent research aimed at setting health standards were used to verify the reliability of the proposed formulas.

The differences between the calculated and the experimentally obtained no-effect chronic toxicity doses in a comparison of 11 organophosphorus compounds did not exceed a factor of 10.

Thus, this method of predicting the parameters of chronic toxicity may be used under certain conditions, but it requires further development and elaboration. To be predictive of the toxicity of substances, the experimental data must be analyzed for relationships with the characteristic physical and chemical properties of the substances, such as indices of bonding energy, electron density distribution of the molecules, with the complex of the substances' toxicological parameters yielding three-member equations, four-member equations, etc. Our experiments further indicate that the use of regression equations for prediction purposes is promising only for certain grops of chemical compounds.

Equations for Predicting Toxicity

It follows from the foregoing material that the use of certain toxicological parameters—specifically, the quantity LD_{50}—to determine the chronic toxicity of substances is not always a reliable criterion (organochlorine compounds). Thus, *the correlation coefficient in some cases was no* more than 0.5. This would appear to be related to the fact that substances with different degrees of cumulative action were included in the analysis. Use of the relationship of LD_{50} values to threshold doses for chronic effect of the compounds, which defines a substance's cumulative effect, may therefore furnish a more reliable approach to prediction. Our analysis yielded a correlation in the order of 10–100 for noncumulative or only slightly cumulative substances, 1000 for moderately cumulative substances, and 10,000–100,000 for highly cumulative substances. These correlations have already found practical applications in research designed to establish the doses of substances that should be used in chronic experiments. In some cases, application of these correlations provided the basis for deciding not to conduct chronic experiments of substances which markedly influence the organoleptic properties of water. Apparently, the organoleptic effect accurately integrates several toxicometric indicators, particularly the single exposure index and the index of the substance's cumulativeness.

Rapid Testing

This last point was one of the bases for the rapid experimental method worked out by us, the main purposes of which are to evaluate the developmental dynamics of the toxicodynamic process in its initial phase and to seek principles in the qualitative characteristics of the substance's impact in subacute and long (chronic) experiments.

The following requirements lie at the foundation of the method we developed (Figs. 4 and 5).

The administered dosages should be below the lethal level. The use of large doses in such experiments is inadvisable, since small, threshold doses are used in chronic experiments, and the size of the dose is a factor of great significance for revealing the different degrees of cumulative effect on substances. Furthermore, large doses leave no chance for the body to develop compensatory (protective) reactions, which develop under actual conditions of intoxication. When large doses are used, the physical accumulation, which in the case of small doses of many substances is of limited importance or even totally lacking, may prevail. It was also found that the optimal dosages for research are those whose effect under the usual conditions of intoxication (daily exposure) is observed in the first few days but virtually disappears after repeated exposure.

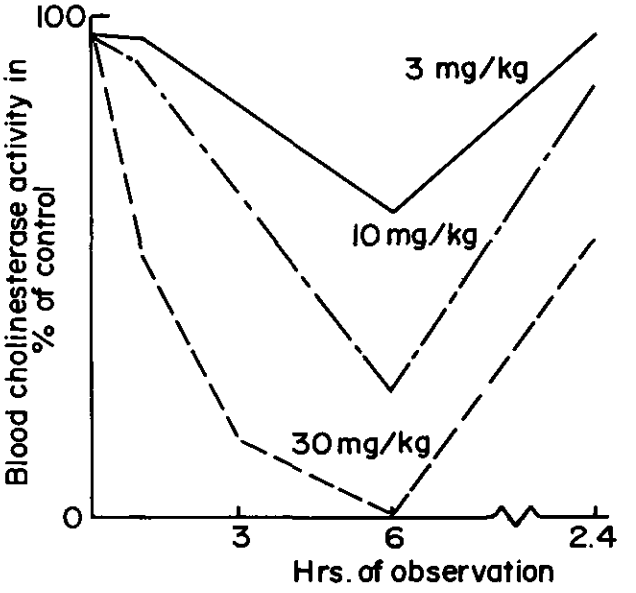

FIGURE 4. Study of cumulative properties of substances by following change in blood cholinesterase activity on hourly basis.

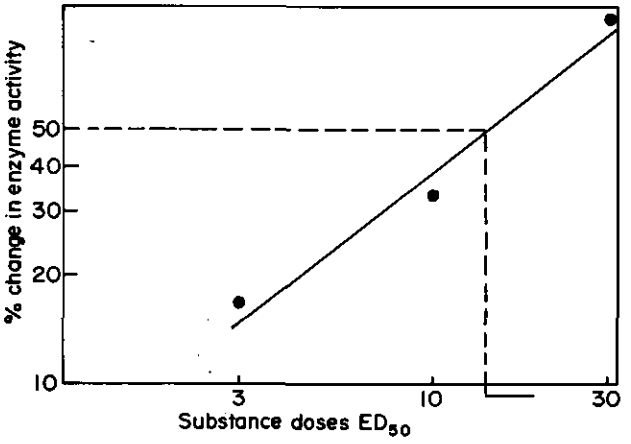

FIGURE 5. Study of cumulative properties of substances by plotting change in enzyme activity. Plot of logarithm of ED_{50} (probability of showing effect) from Fig. 4.

Constant administration of small doses of a substance requires attention only to the graduated effect, i.e., consideration of the changes in functional effects as a function of the substance's toxicodynamics.

The intoxication should be monitored both by the hour, for example 1, 3, 6, 12, and 24 hr after administration of the substance, and by the day, for example on days 1, 5, 10, 15, 20, and 30. In the latter case the graduated indicator is determined on the basis of the time of maximum change in the observed function during a 24-hr period.

Both the hourly and the daily observation results should be reduced to an expression of quantitative probability of the ED_{50} type, (average effective doses), ascertained according to the dose-effect principle. The isoeffective quantities obtained in this way for different time periods of observation ($ED_{50}\,p$) permit conversion to the dose–time relationship, which can be recorded as an isoeffective probability curve. The advantage of this method of expressing the results of an experiment is that it makes possible the graphic presentation of the relationship between the processes of functional breakdown and the adaptive response reactions of an organism along with the prevalence of one of these processes or their phases. The graphic analysis of these types of reactions of an organism for the initial stage of intoxication makes it possible to predict their future course along with the chronic toxicity parameters of substances. Naturally the processing of materials for obtaining such indices using the probit analysis method requires test verification of three to four doses.

The toxicodynamics of a substance in short-term experiments should be studied for no less than 15 days, since the toxic process develops in phases in the early stages of intoxication (initial decompensation and physiological adaptation). The optimum time frame is 20–30 days, with the researcher determining when to end the experiment on the basis of the dynamics of the developing process.

Taking all these factors into account, we studied nine organophosphate compounds in our research: methylnitrophos, acetophos, coral, chlorophos, methylacetophos, sulfidophos, isophos-2, trichlormetaphos, ricid). The experiments showed that the derived isoeffective probability curves faithfully reflect the development of the first phase of intoxication. A graphic analysis of the results reveals a general phenomenon namely: a rise of dose required for intoxication (decline in the value of ED_{50}) by days 5 and 15 with gradual restoration of the depressed enzyme's activity by day 20 or 30; with further observation, the relationship of the processes of intoxication and adaptation remains practically constant, i.e., the activity of the enzyme reaches a state of equilibrium at some level, usually below that characteristic of healthy animals. In some cases, the restoration of cholinesterase activity under organophosphate intoxication returns almost to the initial level after 20–30 days (ricid, methylacetophos), and when some compounds are administered (Fig. 6), the processes of adaptation definitely prevail over the processes of intoxication beginning in the first days and continuing to the end of observation (chlorophos).

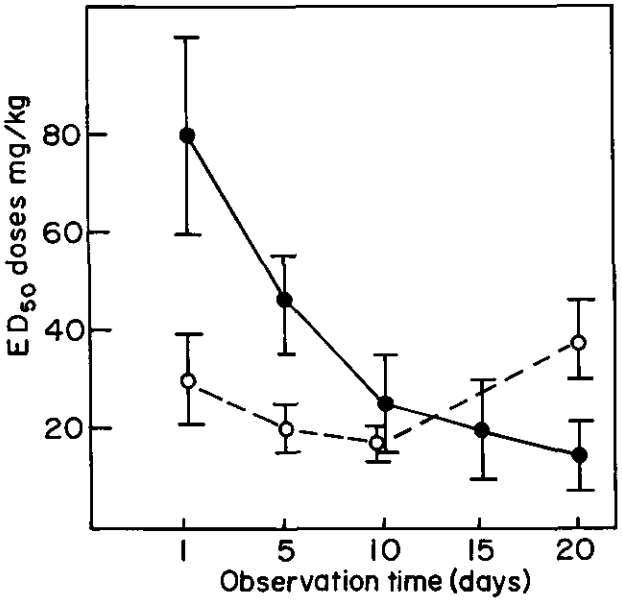

FIGURE 6. Toxicodynamics of substances in short-term experiments: (○) chlorofos; (●) methylnitrophos.

What criteria can be used for evaluating the curves from a 20- to 30-day experiment in developing approaches and recommendations for the prediction of the chronic toxicity of substances?

To use the short-term experiments for predictive purposes portraying the principles of the toxicodynamic process by a simple approximation of a straight-line equation, is difficult in this case. If the segment of the curve corresponding to 10–15 days of intoxication can be reflected to some extent as a parabolic function of the type $x=ay^b$ or a straight-line equation of the general type $\log x = \log a + b \log y$, this relationship may not be used for prediction purposes on the basis of a 20–30-day experiment, because the direction of the curves begins to

change at day 10 to 15. It is not surprising that Hayes (*1*), in his attempt to predict of the cumulative properties of substances by recording the time of death of animals during 1000 days of observation, was able to delineate only that part of the curve in the span from day 6 to day 15 of the entire research period. It appears that, to express such curves, only an equation with three or more members, which takes fuller account of the developing toxicodynamic picture, can be used. The selection of appropriate functions is somewhat difficult, and this problem we feel can be solved through the accumulation of more comprehensive data.

In view of the fact that a deepening of the intoxication process is lacking during protracted observation (beyond 20–30 days) for all of the substances, we used an empirical approach for predicting the chronic toxicity of substances. Our analysis of the data obtained in short-term experiments revealed that the ED_{50} values found on days 20 to 30 of observation and the analogous values found for months 6 to 10 of research for 17 organophosphate compounds differed by one order of magnitude, while the differences between the values for ED_{50} over months 6 to 10 of intoxication and the chronic threshold doses range within the fivefold limits. Considering these correlations and the various requirements of the method developed for conducting short term experiments, we calculated the parameters of chronic toxicity for a number of organophosphate compounds for a long-term 6 month experiment. Different approaches were used for verifying the reliability of the estimated doses. First, for four of the substances studied (ricid, sulfidophos, isophos-2, and methylnitrophos), a full-fledged chronic experiment was conducted, and the threshold and no-effect doses were ascertained. The estimated and experimentally ascertained values differed only by a factor of 2 to 4.

Secondly, similarly limited discrepancies were found between the no-effect doses estimated for substances on the basis of our short-term experiments and the results of long-term experiments conducted by other authors. Thirdly, using eqs. (4)–(6) we developed for organophosphate substances, we derived values for threshold doses and compared them with doses predicted by us on the basis of short-term experiments. The discrepancies between these doses did not exceed a factor of 2–3 except in the case of two substances (intrathion, phosphamid), where they reached a factor of 10.

The results of verifying research thus indicate that it is possible, by using rapid methods (short-term experiments) to determine with some precision the threshold and no-effect doses of chronic toxicity for substances (within the limits of normal experimental error).

Prediction of Toxicity by Extrapolation from Simple Biological Organisms

We also studied the question of the possibility of using lower animals as test subjects for purposes of predicting the toxicity of substances for higher animals. Our analysis revealed that the coefficients of correlation between the toxicity indicators for rats and daphnia, fish, and amphibians are 0.52, 0.49, and 0.619; or compared to birds and humans correlation coefficients are 0.82 and 0.86. Furthermore, the coefficients of correlation r between the values for fishery and health standards are even lower (0.35). A weak correlation, $r = 0.4$ was found between the no-effect concentrations for the human body and the MPC for fish. No correspondence was found between the toxicity of substances for mammals and that for water saprophytes (bacteria). This coefficient of correlation equalled 0.15.

Obviously the metabolic characteristics of the toxic action of a substance in lower animals are apparently quite different from those in higher animals. In our opinion, research along these lines is not very promising, and caution is indicated in the use of lower animals to predict the parameters of toxicity of substances for higher animals, including man.

Prediction of Toxicity by Homology

The principles of biological action within homologous series may provide another approach to the prediction of the toxicity of substances. For the example of the series of homologous higher aliphatic alcohols, the principles of homology were shown to extend beyond the values for threshold doses established in chronic experiments (*2*). This enabled the authors to extrapolate within the confines of a homologous series and to estimate the threshold doses for those alcohols which were not subjected to verification in chronic experiments (ethyl, butyl, and decyl).

Thus, the possibility of utilizing the principle of homology in such series should be borne in mind, and cases of individual empirical observations can assist in prediction within the confines of a

homologous series. This line of investigation merits attention, and it would be worthwhile looking into the possibilities of homology and isomerism for other more complex groups of chemical compounds.

On reviewing the proposed methods of prediction in terms of speeding up research to establish threshold and no-effect doses for the chronic experiments on which health standards are based, we conclude that the optimal solution to this problem must lie in the use of several approaches. In our opinion, the most promising directions, all requiring further investigation, are mathematical methods and rapid experimental methods for predicting the chronic toxicity of substances on the basis of short-term experiments by using a probability curve graph constructed on the basis of the three-coordinate dose–effect–time system.

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